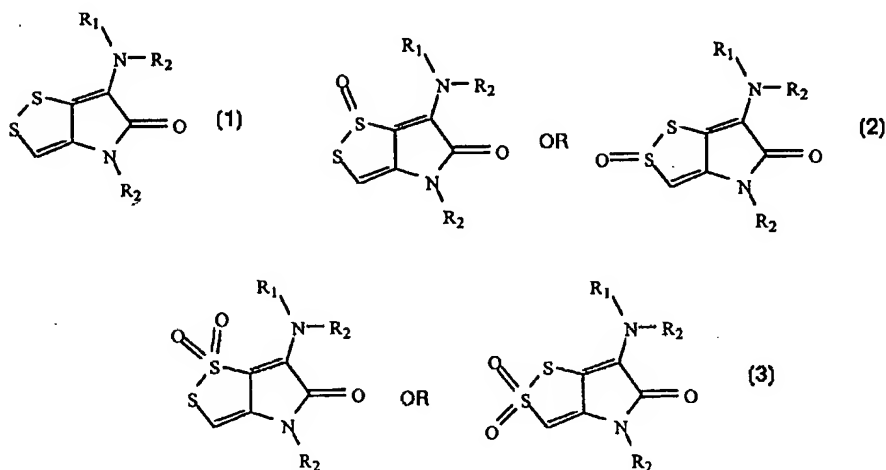




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 31/40, C07D 495/04 // C12P 17/18, C07D 495/04, (C07D 495/04, 339:00, 209:00)		A1	(11) International Publication Number: WO 99/12543
			(43) International Publication Date: 18 March 1999 (18.03.99)
(21) International Application Number: PCT/CA98/00841		(81) Designated States: AU, BG, BR, CH, CN, CZ, DE, ES, GB, HR, HU, ID, IL, JP, KP, KR, LU, MX, NO, NZ, PL, PT, RU, SE, SG, TR, UA, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(22) International Filing Date: 3 September 1998 (03.09.98)			
(30) Priority Data: 2,212,237 5 September 1997 (05.09.97) CA			
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(54) Title: DITHIOLOPYRROLONES AND THEIR CORRESPONDING MONOXIDES AND DIOXIDES AS ANTINEOPLASTIC AGENTS



(57) Abstract

Compounds, isolated from the bacteria *Xenorhabdus bovienii*, having formula (1), wherein R₁ = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, or heterocyclyl group; R₂ = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, heterocyclyl or group acyl; R₃ = hydrogen, alkyl, cycloalkyl, aralkyl, aryl or heterocyclyl group, or (2), wherein R₁ = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, or heterocyclyl group; R₂ = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, heterocyclyl or group acyl; R₃ = hydrogen, alkyl, cycloalkyl, aralkyl, aryl or heterocyclyl group, or (3), wherein R₁ = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, or heterocyclyl group; R₂ = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, heterocyclyl or group acyl; R₃ = hydrogen, alkyl, cycloalkyl, aralkyl, aryl or heterocyclyl group or the salts thereof have antineoplastic activity. The invention provides pharmaceutical compositions containing the compounds and the methods for employing them as medicaments, particularly in the treatment of human and animal cancers.

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**DITHIOLOPYRROLONES AND THEIR CORRESPONDING MONOXIDES AND DIOXIDES AS
ANTINEOPLASTIC AGENTS**

DESCRIPTION OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates to dithiolopyrrolone derivatives, their corresponding monoxides (xenomins) and dioxides (xenorxides) having antineoplastic activities. The present invention also provides antineoplastic compositions comprising these compounds, the salts thereof, and methods of using the inventive compounds as antineoplastic agents.

SUMMARY OF THE INVENTION

The present invention provides dithiolopyrrolone derivatives, their corresponding monoxides (xenomins) and dioxides (xenorxides) having antineoplastic activities. The present invention also provides antineoplastic compositions comprising these compounds, the salts thereof, and methods of using the inventive compounds as antineoplastic agents.

BRIEF DESCRIPTION OF THE DRAWINGS

The following Figure 1, Figure 2 and Figure 3 represent the structural formula of dithiolopyrrolones, their corresponding monoxides (xenomins) and dioxides (xenorxides), respectively.

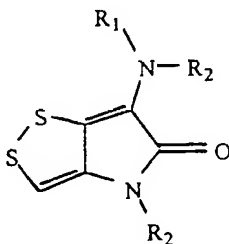


Figure 1

wherein R_1 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, or heterocyclyl group; R_2 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, heterocyclyl or group acyl; R_3 = hydrogen, alkyl, cycloalkyl, aralkyl, aryl or heterocyclyl group.

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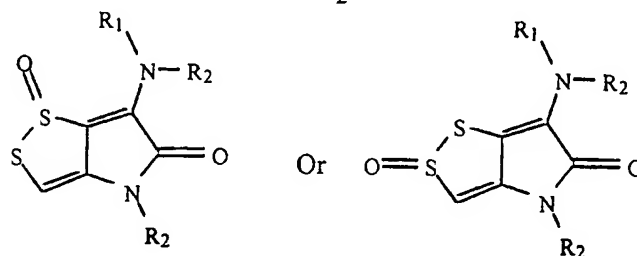


Figure 2

wherein R_1 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, or heterocyclyl group; R_2 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, heterocyclyl or group acyl; R_3 = hydrogen, alkyl, cycloalkyl, aralkyl, aryl or heterocyclyl group.

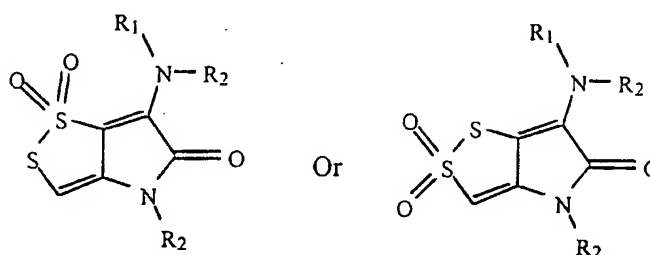


Figure 3

wherein R_1 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, or heterocyclyl group; R_2 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, heterocyclyl or group acyl; R_3 = hydrogen, alkyl, cycloalkyl, aralkyl, aryl or heterocyclyl group.

BACKGROUND

Cancer is one of the major causes of death in humans and animals. Millions of people in the world are diagnosed every year as having cancer, and a large proportion of these people subsequently die of cancer. Although large effort has been made, cancers remain to be hard-to-treat diseases. There continues to be an urgent need for effective anticancer drugs.

Soil organisms have been a traditional source of bioactive materials for the pharmaceutical and agrochemical industry. One of the recent developments has been the commercialization of a soil-living nematode-bacteria combination as biological control agents of insect pests. A crucial feature of this biocontrol agent is that the bacterial symbiont (*Xenorhabdus* spp. or *Photorhabdus* spp.) of the nematode produces a wide range of bioactive metabolites and some of these specific compounds have been isolated, identified and their structures elucidated (Forst and Neilson, 1996). Among these identified compounds, several are dithiopyrrolone derivatives. Dithiopyrrolones were initially

isolated from *Streptomyces* in the 40s and have since then been isolated from other organisms. These compounds include aureothricin, thiolutin, holomycin and xenorhabdins (Hamao *et al.*, 1948; Eisenman *et al.*, 1953; Celmer and Solomons, 1955; von Daehne *et al.*, 1969; McInerney *et al.*, 1991). These compounds possess antimicrobial activity against a wide range of micro-organisms. Recently, new antimicrobial substances, namely xenorxides (Webster *et al.*, 1996), also were found from the bacterial cultures of *Xenorhabdus* spp. As a result of part of the ongoing exploration of the potential usefulness of these metabolites of *Xenorhabdus*, the xenorxides, xenomins and the dithiopyrrolones have been found to be highly active against animal and human cancer cells, and is the subject of this invention.

Animal cells normally undergo various enzymatic and biochemical changes when influenced by various chemical factors. For example, when animal cells were challenged by carcinogens many enzymatic and biochemical activities can be promoted and some of the promoted activities can be experimentally detected. Among dithiopyrrolones and their derivatives, thiolutin is the one that has been studied extensively. Thiolutin inhibits RNA polymerase synthesis (Jimenez *et al.*, 1973; Tipper, 1973) in yeast, has membrane stabilization activity and inhibit platelet aggregation (Yasuyuki *et al.*, 1980) in rat. It has been reported (Menta and Moon, 1991; Sharma *et al.*, 1994; Arnold *et al.*, 1995) that thiolutin inhibited the promotion of several such activities that are possibly related to carcinogenesis in mammary cells that have been exposed to carcinogens. This suggested that thiolutin might be involved in the initiation of cell transformation or the progression of premalignant cells becoming malignant cancer cells. However, this activity has not experimentally established. Although the study of dithiopyrrolones has been on-going for about 50 years, the anticancer activity of thiolutin and other dithiopyrrolones against mammary cells has never been evaluated and reported until now. Xenorxides and xenomins have been discovered very recently, and there is no report in the prior art on their anticancer activity.

DETAILED DESCRIPTION OF THE INVENTION

Although the compounds of the present invention may be synthesized chemically, the initial compounds were obtained from micro-organisms. The organisms used for this invention include *Xenorhabdus bovienii*, a symbiotic bacterium associated with entomopathogenic nematodes. *X. bovienii* and its nematode symbiont *Steinernema feltiae*, used in this invention, were collected from soil in British Columbia, Canada. Briefly, last instar larvae of the Greater Wax Moth, *Galleria mellonella*, were infected with infective juvenile (IJ) nematodes, carrying the *X. bovienii* A21 strain, at a rate of 25 IJs/larvae. After 24 to 48 h the dead insect larvae were surface disinfected by dipping them into 95% EtOH and igniting them. The cadavers were aseptically dissected, haemolymph was streaked onto an

agar culture medium and incubated in the dark at room temperature. The agar medium has the following composition in one litre of distilled water:

beef extract	3 g
peptone	5 g
bromothymol blue	0.025 g
2,3,5-triphenyltetrazolium	0.04 g
Agar	15 g

Sterilized at 121 °C for 15 minutes.

The resulting primary form of *X. bovienii* was maintained and subcultured at 14 d intervals. For consistency, 14% sucrose lyophilized powder of the bacteria stored at -20°C was frequently used as the starting material for cultures. Cultures of *X. bovienii* A21 strain from which the inventive compounds are obtained exhibit the characteristics listed in Table 1 and Table 2:

Table 1. Biochemical properties of *Xenorhabdus bovienii* A21 strain.

Gram reaction	-*
Cell size (µm)	5.3×2.2
Mobility	+
Cell peritrichous	+
Pigmentation	yellow
Catalase	-
Oxidase	-
Urease	-
Lecithinase	+
Lipase(Tween 80)	+

* + positive; - negative.

Table 2. Acid production and utilization of carbon sources by *Xenorhabdus bovienii* A21 strain.

Acid production*		Utilization of carbon sources	
D-Arabinose	+/-	Asparagine	+
Esculine	-	Cystine	-
D-Fructose	+	Glycine	-
D-Galactose	-	Tyrosine	+
D-Glucose	+	Nicotinic acid	-

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Inositol	+/-	Ethanol	-
Inulin	-	Methanol	-
D-Lactose	-	Inositol	+/-
D-Maltose	+	Mannose	+
D-Mannitol	-	D-Galactose	-
D-Mannose	+	D-Glucose	+
D-Raffinose	-	D-Lactose	-
D-Sorbitol	+/-	D-Mannitol	-
L-Sorbose	-	D-Sorbitol	-
D-xylose	-	Ribose	+

* + positive; +/-: weakly positive; - negative.

These characteristics are in agreement with those described for *X. bovienii* by Akhurst, R. J. and N. E. Boemare (1988), and, therefore, establishes the identity of the organism as *X. bovienii*. The bacterial strain from which the compounds of this invention were isolated was deposited under the Budapest Treaty in the American Type Culture Collection (ATCC), Rockville, MD with a deposition number of ATCC 55743.

Cultivation of the microorganism *X. bovienii* yields bioactive substances, xenomins, xenorxides and dithiolopyrrolones. *X. bovienii* may be cultivated (fermented), for example, at about 25 °C under submerged aerobic conditions in an aqueous, nutrient medium containing assimilable carbon (carbohydrate) and nitrogen sources to obtain xenomins, xenorxides and dithiolopyrrolones. The fermentation may be carried out for a time period such as approximately 48 to 96 hours, at the end of which time these compounds have been formed, and may be isolated from the fermentation medium and purified.

After the fermentation has been completed, the fermented broth may be filtered or centrifuged and the pH of the filtrate adjusted to about 7.0 by the addition of hydrochloric acid or kept as it is. The filtrate may then be extracted with a water immiscible, organic solvent, for example, with ethyl acetate or chloroform. The combined organic layers (e.g. pooled ethyl acetate or chloroform extracts) may be concentrated under vacuum (e.g., at about 30 °C) to an oily residue. The oily residue may be mixed with a small amount of organic solvent and chromatographed on a silica gel column. After introduction of the sample, chloroform or other organic solvent may be applied to elute the bioactive fraction. The bioactive fraction may be purified further by high performance, liquid chromatography (HPLC) with organic and/or aqueous solution.

In a conventional culture, the major compounds produced by *X. bovienii* are dithiolopyrrolones while xenorxides and xenomins are present in relatively small amounts. Alternatively, dithiolopyrrolones may be produced from other micro-organisms, by chemical method(s) and/or a combination of both approaches.

The monoxides and dioxides of the corresponding dithiolopyrrolones isolated from *X. bovienii*, namely, xenorxides and xenomins, respectively may be transformed from their corresponding dithiolopyrrolone derivatives by biological or chemical means. By biological means, the culture broth of *X. bovienii*, with the corresponding dithiolopyrrolone derivatives present, may be filtered or centrifuged. The cell-free filtrate may be open to the air for extended periods from one week up to one month with or without stirring at room temperature or at other temperatures. This process may oxidize all or part of the corresponding dithiolopyrrolone derivatives to xenomins and xenorxides. Xenorxides and xenomins may be obtained by oxidation of the corresponding dithiolopyrrolone derivatives by chemical means. The pure dithiolopyrrolone derivatives or their mixture may be dissolved in a mixture of acetone and water, then oxidizing reagents such as potassium peroxymonosulfate and potassium bicarbonate may be added to the mixture. The mixture may be allowed to react for a period of several minutes up to more than one hour. The reaction mixture may be mixed with water, and extracted with organic solvent. The extracts may be combined, dried and purified by column chromatography or HPLC to obtain the corresponding xenorxides and/or xenomins. Additional monoxides and dioxides of the dithiolopyrrolones may be obtained from different microorganisms, synthesized chemically and/or produced by a combination of biological and chemical methods, and several examples are disclosed (Takahashi, *et al.*, 1995; Fujimoto, *et al.* 1996).

The compounds of the present invention include dithiolopyrrolones, their corresponding monoxides such as xenomins, their corresponding dioxides such as xenorxides, and salts thereof. The term "salts", as used herein, denotes acidic and/or basic salts, formed with inorganic and/or organic acids and bases. Suitable acids include, for example, hydrochloric, sulfuric, nitric, benzenesulfonic, acetic, maleic, tartaric and the like which are pharmaceutically acceptable. It is well known to one skilled in the art that an appropriate salt is chosen based on physical and chemical stability, flowability, hygroscopicity and solubility. While pharmaceutically acceptable salts are preferred, particularly when employing the compounds of the invention as medicaments, other salts find utility, for example, in processing these compounds, or where non-medicament-type uses are contemplated.

The antineoplastic agents and use thereof.

The present invention relates to pharmaceutical preparations which contain an active ingredient of these compounds or a pharmaceutically acceptable salt thereof. The dosage form and mode of

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administration, as well as the dosage amount, may be selected by the skilled artisan. Exemplary daily dosages for an adult human are those within the range of about 2.5 mg to about 2,000 mg/day. Administration to a mammalian host may, for example, be oral, parenteral, or topical.

When these compounds or the salts thereof are used as therapeutics, they can be administered alone or in a pharmaceutically suitable formulation containing, in addition to the active ingredient, one or more conventional carriers. Depending on the nature of the disease and/or route of administration, the composition of this invention can be formulated by known means.

Examples of pharmaceutical compositions include any solid (tablets, pills, capsules, granules, powder etc.) or liquid (solutions, suspensions or emulsions) compositions suitable for oral, topical or parenteral administration, and they may contain the pure compound or a salt thereof or in combination with any carrier or other pharmaceutically active compounds. These compositions may need to be sterile when administered parenterally.

The therapeutic compositions of the present invention being employed as antineoplastic agents for treatment of animal and human illness can be easily prepared in such unit dosage form with the employment of pharmaceutical materials which themselves are available in the art and can be prepared by established procedures. The appropriate solid or liquid vehicle or diluent may be selected, and the compositions prepared, by methods known to the skilled artisan. The administration of any of the inventive compound and/or its pharmacologically active and physiologically compatible derivatives is useful for treating animals or humans having a neoplastic disease, for example, colon cancer, cervical cancer, breast cancer, leukemia, lung cancer, ovarian cancer, CNS cancer, renal cancer, prostate cancer, *etc.* using the accepted protocols of the National Cancer Institute (NCI). The dosage administered will be dependent upon the identity of the neoplastic disease, the type of host involved including its age, health and weight, the kind of concurrent treatment, if any, and the frequency of treatment and therapeutic ratio. Illustratively, dosage levels of the administered active ingredients are intravenous, 0.1 to about 200 mg/kg; intramuscular, 1 to about 500 mg/kg and orally, 5 to about 1000 mg/kg of host body weight. Expressed in terms of concentration, an active ingredient can be present in the compositions of the present invention for localized use in a concentration from about 0.01 to about 50% w/w of the composition, preferably about 1 to about 20% w/w of the composition, and for parenteral use in a concentration of from about 0.05 to about 50% w/v of the composition and preferably from about 5 to about 20% w/v. The active ingredients to be employed as antineoplastic agents, can be easily prepared in such unit dosage form with the employment of pharmaceutical materials which themselves are available in the art and can be prepared by established procedures.

EXAMPLE 1. preparation of xenorxides, xenomins and the dithiolopyrrolones

A. Preparation of selected dithiolopyrrolones.

Several dithiolopyrrolones have been reported so far, these include aureothricin, thiolutin, holomycin, xenorhabdins and thiomarinol. Aureothricin was initially reported by Umezawa *et al.* (1948), the structure were fully disclosed by Celmer and Solomons (1955). Thiolutin is produced by several bacterial species including *Streptoverticillium album*, which is available from the American Type Culture Collection(ATCC), Rockville, MD with an ATCC number of 33049. The production of thiolutin and holomycin by chemical synthesis was published by Schmidt and Geiger (1962), Hagio, K. and Yoneda, N. (1974) and Stachel, H.D., *et al.* (1992). Production of xenorhabdins was reported and fully reported by McInerney *et al.* (1991) and by Li *et al.* (1995). The preparation of additional different compounds with the dithiolopyrrolone ring was recently disclosed by Baggaley *et al.* (1994a, b) and by Takahashi *et al.* (1994). The dithiolopyrrolones used for the subject invention are prepared by the methods described in the cited references, and the structure of each dithiolopyrrolone derivative confirmed by its NMR spectroscopy. Skilled chemists will be able to use procedures as disclosed herein, and others, to obtain these dithiolopyrrolones from available stock substances. In carrying out such operations, any suitable filtration, chromatographic, and other purification techniques might be employed by those skilled in the art. It will be apparent to those skilled in the art that materials and reagents to carry out such operations are commercially available from chemical companies, so no details are given respecting them.

B. Preparation of the corresponding dioxides from dithiolopyrrolones by microbial fermentation.

Xenorxides: Cultures of *X. bovienii* were shaken at 180 rpm on an Eberbach gyrorotary shaker for 24 h at 25 °C . Bacterial fermentation was initiated by adding 100 ml of this bacterial culture to 900 ml of tryptic soy broth in a 2,000 ml flask. The flask was incubated in the dark at 25 °C on a gyrorotary shaker. After 96 h, the culture was immediately centrifuged (12,000 g, 20 minutes, 4 °C) to separate the bacterial cells. The cell-free broth was then extracted with ethyl acetate four times. The combined extracts were dried with anhydrous sodium sulfate and then filtered through filter paper. The filtrate was concentrated on a rotary evaporator below 30 °C , under vacuum to yield a brown oil. After the above experiment was repeated 10 times, approximately 3 g of the oil was obtained. The crude extracts were then loaded onto a silica gel (200 g silica gel 60, 40 cm x 5 cm, EM Science, Darmstadt, Germany) chromatographic column. The yellow bioactive fraction was eluted out with ether or ethyl acetate. This bioactive fraction was then subject to HPLC on a C₁₈ preparative column (Spherisorb 10 (ODS(1)), 250

X 10 mm, 10 micro, Phenomenex, Torrance, CA) with a program (isocratic at 10% acetonitrile in water for 5 min, then gradually increasing to 85% acetonitrile in 35 min, isocratic for 5 min, then decreasing back to 10% in 2 min) at 2.5 ml/min. The eluate was monitored at 254 nm.

XENORXIDE 1 (about 0.3 mg per liter of the culture broth) was eluted at 33.6 min, and this was followed by XENORXIDE 2 (0.2 mg/l) which was eluted at 35.2 min.

C. Preparation of the corresponding monoxides from dithiolopyrrolones by microbial fermentation.

Xenomins: For the purification of xenomins, the culture and extraction conditions were the same as for xenorxides. After the extraction, the concentrated crude extract was processed through a silica gel chromatographic column with ethyl acetate as the eluent. After the less polar bioactive material was eluted, the more polar bioactive fraction was obtained by eluting with methanol. The more polar bioactive fraction was concentrated under vacuum, and separated by a C_{18} chromatographic column first with water as eluent, then 25% methanol in water, 50% methanol, 75% methanol and finally, pure methanol. The most bioactive fraction was eluted with 75% methanol in water. This bioactive fraction was then concentrated and separated by HPLC on a C_{18} preparative column (Spherisorb 10 (ODS(1)), 250 × 10 mm, 10 micro, Phenomenex, Torrance, CA) with a program (30% MeCN in H_2O for 1 min and gradually increasing to 70% MeCN in H_2O in 24 min, isocratic for 5 min) at 2.0 ml/min. The eluate was monitored at 254 nm. Active peak 1 (25.4 min) and peak 2 (25.8 min) were collected. Active peak 1 was concentrated, and further separated by preparative silica gel TLC with 60% ethyl acetate in dichloromethane as the eluent to give xenomin 1 ($R_f = 0.32$). Active peak 2 was concentrated, and further separated by preparative silica gel TLC with 60% ethyl acetate in dichloromethane as the eluent to give xenomin 2 ($R_f = 0.31$).

D. Preparation of xenorxides from their corresponding dithiolopyrrolone derivatives by biological transformation.

The cell-free broth was obtained using the same method as described above, and was then stored at 4 °C to room temperature for 3 to 6 weeks. Then the aqueous broth was extracted with ethyl acetate, and the combined extracts were separated using the same process discussed above. XENORXIDE 1 was eluted at 33.6 min (2 mg/l), XENORXIDE 2 was eluted at 35.2 min (1.5 mg/l).

E. Preparation of the corresponding dioxides from dithiolopyrrolones by chemical oxidation.

One hundred and fifty two mg of 6-(hexanamido)-4-methyl-4,5-dihydro-1,2-dithiolo[4,3-b]pyrrol-5-one (XN1) were dissolved in a mixture of 20 ml of acetone and 15 ml of water, and then cooled to 0 °C with ice. Potassium peroxymonosulfate (510 mg) was added to the solution at 0 °C. The reaction mixture was stirred for one hour at 0 °C. Then 5 ml of saturated solution of potassium bicarbonate was added to the reaction mixture with continuous stirring for 0.5 h at 0 °C. The reaction

mixture, after water was added, was extracted with ethyl acetate three times. The extracts were combined, dried over Na_2SO_4 and evaporated to give the crude product, which was then purified by silica gel column chromatography with hexane: ethyl acetate (2:1) to give pure xenorxide 1 (78 mg, 51% yield). Similarly, xenorxide 3 was produced from 6-(5'-methylhexanamido)-4,5-dihydro-1,2-dithiolo[4,3-b]pyrrol-5-one with 50% yield.

Example 2. Identification of the active components

NMR spectra were recorded on a Bruker WM400 spectrometer in CDCl_3 , using residual CDCl_3 (~7.25) as internal standard. Low resolution mass spectra were obtained on a Hewlett-Packard 5985B GC/MS system operating at 70 eV using a direct probe. High resolution MS spectra were recorded on a Kratos MS80 instrument. IR spectra were recorded as neat film on NaCl using a Perkin-Elmer 599B spectrometer. (Abbreviations used as follows: EI = Electron Impact, M = Molecular Ion. t = triplet, J = coupling constant, Hz = Hertz, d = doublet, m = multiplet, bs = broad singlet).

6-(Acetamido)-4-methyl-4,5-dihydro-1,2-dithiolo[4,3-b]pyrrol-5-one (Thiolutin) (XN0): $^1\text{HNMR}$ ($\text{DMSO}-d_6$) δ 2.05 (3H, s), 3.92 (3H, s), 7.19 (1H, s) and 9.85 (1H, broad s); EIMS m/e 228 (M^+), 186.

6-(Hexanamido)-4-methyl-4,5-dihydro-1,2-dithiolo[4,3-b]pyrrol-5-one (XN1): $^1\text{HNMR}$ (CDCl_3) δ 0.90 (3H, t, $J=6.9\text{Hz}$), 1.35 (4H, m), 1.70 (2H, m), 2.35 (2H, t, $J=7.4\text{Hz}$), 3.35 (3H, s), 6.63 (1H, s) and 7.43 (1H, broad s); EIMS m/e 284 (M^+), 186.

6-(5'-Methylhexanamido)-4,5-dihydro-1,2-dithiolo[4,3-b]pyrrol-5-one (XN3): $^1\text{HNMR}$ (CDCl_3) δ 0.89 (6H, d, $J=6.6\text{Hz}$), 1.24 (3H, m), 1.70 (2H, m), 2.32 (2H, t, $J=7.6\text{Hz}$), 6.74 (1H, s), 7.44 (1H, broad s) and 7.94 (1H, broad s); EIMS m/e 284 (M^+), 172.

XENORXIDE 1 (XO1): EIMS: 317(2), 316(M^+ , 13), 220(9), 219(9), 218(100), 186(23), 154(16), 99(40), 71(39); HRMS: 316.0555 (Calc. for $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_4\text{S}_2$: 316.0551, 20), 217.9824 (Calc. for $\text{C}_6\text{H}_6\text{N}_2\text{O}_3\text{S}_2$: 217.9820, 100), 154.0197 (Calc. for $\text{C}_6\text{H}_6\text{N}_2\text{OS}$: 154.0201, 16); IR (KBr): 3448, 3298, 3275, 1720, 1686, 1654, 1637, 1560, 1522, 1310, 1139, 551 cm^{-1} ; $^1\text{HNMR}$ (CDCl_3) δ : 7.56 (1H, bs, CO-NH), 6.35 (1H, s, H-3), 3.20 (3H, s, N-Me), 2.38 (2H, t, CO-CH₂, $J=7.4\text{Hz}$), 1.67 (2H, m, CH₂), 1.32 (4H, m, CH₂CH₂), 0.89 (3H, t, $J=7.0\text{Hz}$); $^{13}\text{CNMR}$ (CDCl_3) δ : 171.6(s, CON), 164.7 (s, CO), 145.4(s, C7), 121.3(s, C6), 116.2(s, C8), 109.2(d, C3), 36.4, 31.2, 27.8, 24.6, 22.3, 13.8.

XENORXIDE 2 (XO2): EIMS: 330(M^+ , 10), 218(100); HRMS: 330.0707 (Calc. for $\text{C}_{13}\text{H}_{18}\text{N}_2\text{O}_4\text{S}_2$: 330.0708, 18), 217.9829 (Calc. for $\text{C}_6\text{H}_6\text{N}_2\text{O}_3\text{S}_2$: 217.9820, 100), 154.0213 (Calc. for $\text{C}_6\text{H}_6\text{N}_2\text{OS}$: 154.0201, 16); IR (KBr): 3438, 3298, 1719, 1686, 1654, 1637, 1560, 1522, 1400,

1310, 1142, 551 cm^{-1} ; ^1H NMR (CDCl_3) δ : 7.56 (1H, bs, CO-NH), 6.35 (1H, s, H-3), 3.20 (3H, s, N-Me), 2.36 (2H, t, CO-CH₂, $J=7.4$ Hz), 1.67 (2H, m, CH₂), 1.2-1.6 (1H, m, CH), 1.22 (2H, m, CH₂), 0.89 (6H, d, $J=6.6$ Hz); Different NOE experiment showed the NOE effect between the peak at 6.35 ppm and 3.20 ppm; ^{13}C NMR (CDCl_3) δ : 171.6(s, CON), 164.7 (s, CO), 145.4(s, C7), 121.3(s, C6), 116.2(s, C8), 109.2(d, C3), 38.2(t, CH₂), 36.7(t, CH₂), 28.0(q, CH₃), 27.8(d, CH), 22.8(t, CH₂), 22.4(q, CH₃).

XENORXIDE 3 (XO3): EIMS: 316(M^+ , 7.5), 218(9), 204(65) 185(7), 172(15), 105 (25), 95(95), 69(100), 43 (70); ^1H NMR (CDCl_3) δ : 10.10 (1H, bs), 9.32 (1H, bs), 6.85 (1H, s, H-3), 2.53 (2H, t, CO-CH₂, $J=7.5$ Hz), 1.66 (2H, m, CH₂), 1.58 (1H, m), 1.21 (2H, m,), 0.87 (6H, d, $J=6.6$ Hz).

Xenomin 1 (XM1): EIMS: 300(M^+ , 13), 202(36), 186(44), 185(41), 85(62), 69(100); HRMS: 300.0605 (Calc. for $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_3\text{S}_2$: 300.0602, 12), 201.9871 (Calc. for $\text{C}_6\text{H}_6\text{N}_2\text{O}_2\text{S}_2$: 201.9871, 30); ^1H NMR (CDCl_3) δ : 7.52 (1H, bs, CO-NH), 6.46 (1H, s, H-3), 3.30 (3H, s, N-Me), 2.49 (2H, t, CO-CH₂, $J=7.4$ Hz), 1.79 (2H, m, CH₂), 1.41 (4H, m, CH₂-CH₂), 0.90 (3H, t, $J=6.9$).

Xenomin 2 (XM2): EIMS: 314(M^+ , 21), 218(15), 202(59), 187(18), 186(98), 185(73), 69(100); HRMS: 314.0759 (Calc. for $\text{C}_{13}\text{H}_{18}\text{N}_2\text{O}_3\text{S}_2$: 314.0759, 12), 201.9871 (Calc. for $\text{C}_6\text{H}_6\text{N}_2\text{O}_2\text{S}_2$: 201.9871, 45); ^1H NMR (CDCl_3) δ : 7.43 (1H, bs, CO-NH), 6.46 (1H, s, H-3), 3.30 (3H, s, N-Me), 2.48 (2H, t, CO-CH₂, $J=7.6$ Hz), 1.71 (2H, m, CH₂), 1.29 (3H, m, CH and CH₂), 0.88 (6H, d, $J=7.0$).

Example 3. Dithiolopyrrolones, the corresponding monoxides and dioxides as antineoplastic agents.

The anticancer activity of a particular compound can be demonstrated by standard assays. The method normally used by the American National Cancer Institute (NCI) for the effectiveness of a compound is based on the LC_{50} . The LC_{50} is the compound concentration at which half of the cancer cell population is killed. The assay is commonly used by those skilled in the art and are accepted as indicative of anticancer activity in mammals. The test animal cancer cells are available from ATCC, NCI and other organizations. The anticancer activities of compounds of the present invention have been determined in cell cultures of various human cancer cells (see table below) using the standard NCI method with slight modifications. The specifications of the procedure was described by Skehan *et al.* (1990). Briefly, cancer cells were grown in RPMI-1640 medium with glutamine and 10% fetal calf serum, and were harvested from exponential-phase maintenance cultures. The harvested cells were counted and dispensed into replicate 96-well culture plates in 180 μl volumes for each well with a cell density of up to 2,500 cells/well. The cells were allowed to settle for about 4 hours at 37 $^\circ\text{C}$. Then 20 μl of medium containing the test compound was added into each well, resulting in a suitable final test compound concentration. The test plates were then incubated at 37 $^\circ\text{C}$. The test was terminated after

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incubation by adding to each well 50 μ l of cold 50% trichloroacetic acid. The cells were fixed for an hour at 4 °C and then washed five times with tap water. The washed plates were air-dried and stained for 30 minutes with 0.4% (wt/vol) sulforhodamine B (SRB) which was dissolved in 1% acetic acid. At the end of the staining period, the SRB was removed and plates were quickly rinsed five times with 1% acetic acid. After being rinsed, the plates were air-dried and 100 μ l of 10 mM tris base (pH 10.5) were added to each well to dissolve the dye bound to the cells. The plates were placed on a gyratory shaker and shaken (100 rpm) for 10 minutes. Finally, the plates were read in a microtiter plate reader at 570 nm. All the compounds tested exhibited very strong anticancer activity against these cancer cells (see table below).

Antineoplastic activity:

Cancer cells	LC ₅₀ (10 ⁻⁴ M)						
	XN0	XN1	XN3	XO1	XO2	XM1	XM2
HT29	0.13	0.11	0.11	0.22	0.85	0.47	0.11
MCF-7	0.35	0.56	0.39	0.89	0.95	0.47	0.55
Hela	0.31	0.46	0.32	0.16	0.13	0.80	0.21
P388	—*	—	—	0.66	0.28	—	—
NCI-H460	—	—	0.03	0.28	0.22	—	—
Sk-Mel-28	—	—	0.24	0.19	0.73	—	—
LNCap	—	—	—	0.54	0.19	—	—
DU-145 (Prostate)	—	—	0.14	1.80	1.60	—	—
UO-31 (Renal cancer)	—	—	0.18	1.85	6.0	—	—
SF-295 (CNS cancer)	—	—	0.04	1.58	2.12	—	—

* not tested.

From the foregoing embodiments and examples, it is apparent that an invention has been herein described and illustrated. While our above description contains many specificities, these should not be construed as limitations on the scope of the invention, but rather as examples of preferred embodiments. Accordingly, the scope of the invention should not be determined by the embodiments presented, but by the appended claims and their legal equivalents.

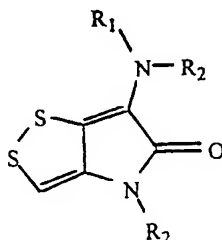
REFERENCES CITED

1. Akhurst, R. J. and N. E. Boemare, 1988. *J. Gen. Microbiol.* Vol. 134, 1835-1845
2. Arnold, J. T. *et al.*, 1995. *Cancer Research* 55:537-543.
3. Baggaley, K.H. *et al.*, 1994a. WO 94/26750.
4. Baggaley, K.H. *et al.*, 1994b. WO 94/28001.
5. Celmer, W. D. and I. A. Solomons 1955. *J. Amer. Chem. Soc.* 77:2861-2865.
6. Eisenman, W. *et al.*, 1953. *Antibiotics and Chemotherapy* 3: 385-392.
7. Forst, S. and K. Nealson, 1996. *Microbiol. Rev.* 60:21-43.
8. Fujimoto, K. *et al.*, 1996. WO 96/23795.
9. Hagio, K. and Yoneda, N. 1974. *Bull. Chem. Soc. Japan.* 47:1484-1489.
10. Jimenez, A. *et al.*, 1973. *Antimicrob. Ag. Chemother.* 729-738.
11. Li, *et al.*, 1995. *Appl. Environ. Microbiol.* 61:4329-4333.
12. Li, J. *et al.*, 1995. *J. Nat. Prod.* 58:1081-1085.
13. McInerney, B. V. *et al.*, 1991. *J. Nat. Prod.* 54:774-784.
14. Menta R. G. and R. C. Moon, 1991. *Anticancer Research* 11:593-596.
15. Monks *et al.*, 1991. *J. Natl. Cancer Inst.* 83:757-766.
16. Ninomiya, Y. T. *et al.*, 1980. *Chem. Pharm. Bull.* 28:3157-3162.
17. Sharma, S. *et al.*, 1994. *Cancer Research* 54:5848-5855.
18. Shroeder *et al.*, 1981. *J. Med. Chem.* 24:1078-1083.
19. Skehan, P. *et al.*, 1990. *J. Natl. Cancer Inst* 82:1107-1118.
20. Stachel, H.D. *et al.* 1992. *Liebigs Ann. Chem.* 473-480.
21. Takahashi S. *et al.*, 1994. US patent. 5,292,892.
22. Takahashi S. *et al.*, 1995. US patent. 5,399,711.
23. Tipper D. J. 1973. *J. Bacteriol.* 116:245-256.
24. Umezawa, H. *et al.* 1948. *Jap. Med. J.* 1:512-517.
25. Webster *et al.* WO: 96/32396.
26. von Daehne, W. *et al.*, 1969. *J. Antibiotics* 22:233-235.

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CLAIMS

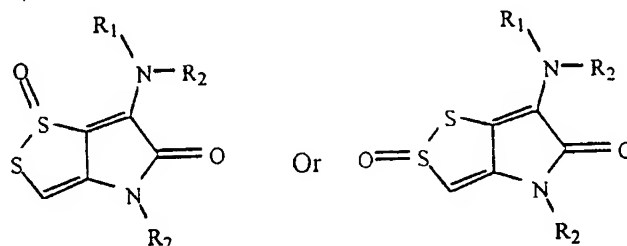
We claim:

1. A pharmaceutical composition for treating neoplastic diseases comprising a compound having the following structure formula



wherein R_1 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, or heterocyclyl group; R_2 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, or heterocyclyl, acyl group; R_3 = hydrogen, alkyl, cycloalkyl, aralkyl, aryl or heterocyclyl group, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutical carrier or dilution.

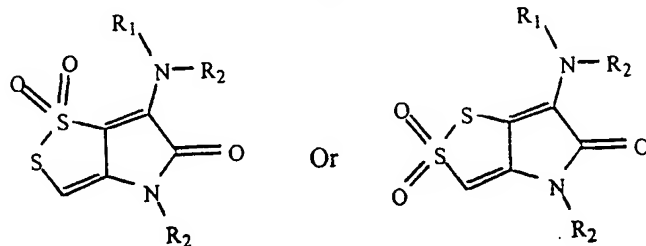
2. A pharmaceutical composition for treating neoplastic diseases comprising a compound having the following structure formula



wherein R_1 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, or heterocyclyl group; R_2 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, or heterocyclyl, acyl group; R_3 = hydrogen, alkyl, cycloalkyl, aralkyl, aryl or heterocyclyl group, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutical carrier or dilution.

3. A pharmaceutical composition for treating neoplastic diseases comprising a compound having the following structure formula

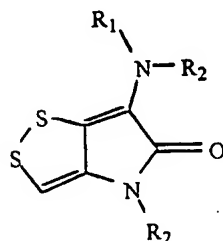
-15-



wherein R_1 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, or heterocyclyl group; R_2 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, or heterocyclyl, acyl group; R_3 = hydrogen, alkyl, cycloalkyl, aralkyl, aryl or heterocyclyl group, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutical carrier or dilution.

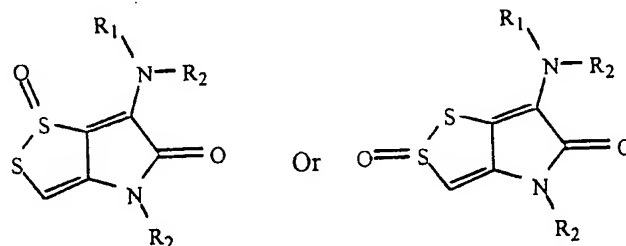
4. A pharmaceutical composition for treating neoplastic diseases comprising a compound of claim 1, wherein R_1 = hydrogen; R_2 = acyl; R_3 = hydrogen, methyl; or a pharmaceutically acceptable salt thereof, in association with a pharmaceutical carrier or dilution.
5. A pharmaceutical composition for treating neoplastic diseases comprising a compound of claim 2, wherein R_1 = hydrogen; R_2 = acyl; R_3 = hydrogen, methyl; or a pharmaceutically acceptable salt thereof, in association with a pharmaceutical carrier or dilution.
6. A pharmaceutical composition for treating neoplastic diseases comprising a compound of claim 3, wherein R_1 = hydrogen; R_2 = acyl; R_3 = hydrogen, methyl; or a pharmaceutically acceptable salt thereof, in association with a pharmaceutical carrier or dilution.
7. A pharmaceutical composition for treating neoplastic diseases comprising a compound of claim 1, wherein R_1 = hydrogen, R_2 = acyl group with a straight or branched one to ten carbon chain; R_3 = hydrogen, or methyl, or a pharmaceutically acceptable salt thereof in association with a pharmaceutical carrier or dilution.
8. A pharmaceutical composition for treating neoplastic diseases comprising a compound of claim 2, wherein R_1 = hydrogen, R_2 = acyl group with a straight or branched one to ten carbon chain; R_3 = hydrogen, or methyl, or a pharmaceutically acceptable salt thereof in association with a pharmaceutical carrier or dilution.
9. A pharmaceutical composition for treating neoplastic diseases comprising a compound of claim 3, wherein R_1 = hydrogen, R_2 = acyl group with a straight or branched one to ten carbon chain; R_3 = hydrogen, or methyl, or a pharmaceutically acceptable salt thereof in association with a pharmaceutical carrier or dilution.

10. A method of inhibiting the growth of mammalian tumors, comprising administering to a subject in need of such treatment, an effective antineoplastic amount of a compound of the structures shown below,



wherein R_1 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, or heterocyclyl group; R_2 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, or heterocyclyl, acyl group; R_3 = hydrogen, alkyl, cycloalkyl, aralkyl, aryl or heterocyclyl group, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutical carrier or dilution.

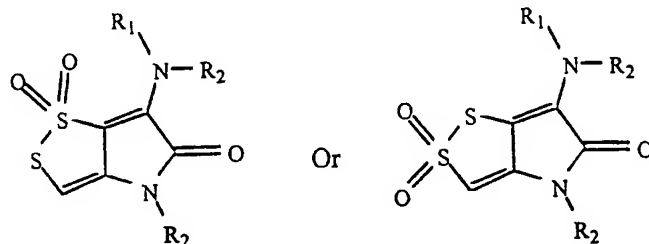
11. A method of inhibiting the growth of mammalian tumors, comprising administering to a subject in need of such treatment, an effective antineoplastic amount of a compound of the structures shown below,



wherein R_1 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, or heterocyclyl group; R_2 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, or heterocyclyl, acyl group; R_3 = hydrogen, alkyl, cycloalkyl, aralkyl, aryl or heterocyclyl group, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutical carrier or dilution.

12. A method of inhibiting the growth of mammalian tumors, comprising administering to a subject in need of such treatment, an effective antineoplastic amount of a compound of the structures shown below,

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wherein R_1 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, or heterocyclyl group; R_2 = hydrogen, substituted or unsubstituted alkyl, cycloalkyl, aryl, aralkyl, or heterocyclyl, acyl group; R_3 = hydrogen, alkyl, cycloalkyl, aralkyl, aryl or heterocyclyl group, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutical carrier or dilution.

13. A method of inhibiting the growth of mammalian tumors, comprising administering to a subject in need of such treatment, an effective antineoplastic amount of a compound of claim 10, wherein R_1 = hydrogen; R_2 = acyl; R_3 = hydrogen, methyl; or a pharmaceutically acceptable salt thereof, in association with a pharmaceutical carrier or dilution.
14. A method of inhibiting the growth of mammalian tumors, comprising administering to a subject in need of such treatment, an effective antineoplastic amount of a compound of claim 11, wherein R_1 = hydrogen; R_2 = acyl; R_3 = hydrogen, methyl; or a pharmaceutically acceptable salt thereof, in association with a pharmaceutical carrier or dilution.
15. A method of inhibiting the growth of mammalian tumors, comprising administering to a subject in need of such treatment, an effective antineoplastic amount of a compound of claim 12, wherein R_1 = hydrogen; R_2 = acyl; R_3 = hydrogen, methyl; or a pharmaceutically acceptable salt thereof, in association with a pharmaceutical carrier or dilution.
16. A method of inhibiting the growth of mammalian tumors, comprising administering to a subject in need of such treatment, an effective antineoplastic amount of a compound of claim 10, wherein R_1 = hydrogen, R_2 = acyl group with a straight or branched one to ten carbon chain; R_3 = hydrogen, or methyl, or a pharmaceutically acceptable salt thereof in association with a pharmaceutical carrier or dilution.
17. A method of inhibiting the growth of mammalian tumors, comprising administering to a subject in need of such treatment, an effective antineoplastic amount of a compound of claim 11, wherein R_1 = hydrogen, R_2 = acyl group with a straight or branched one to ten carbon chain; R_3 = hydrogen, or methyl, or a pharmaceutically acceptable salt thereof in association with a pharmaceutical carrier or dilution.

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18. A method of inhibiting the growth of mammalian tumors, comprising administering to a subject in need of such treatment, an effective antineoplastic amount of a compound of claim 12, wherein R_1 = hydrogen, R_2 = acyl group with a straight or branched one to ten carbon chain; R_3 = hydrogen, or methyl, or a pharmaceutically acceptable salt thereof in association with a pharmaceutical carrier or dilution.

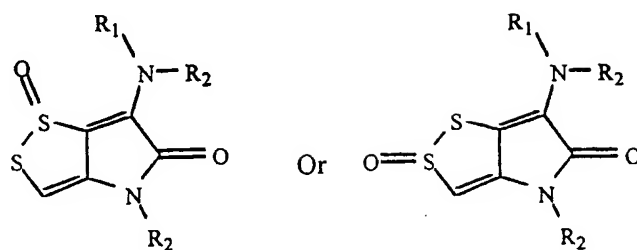


Figure 2

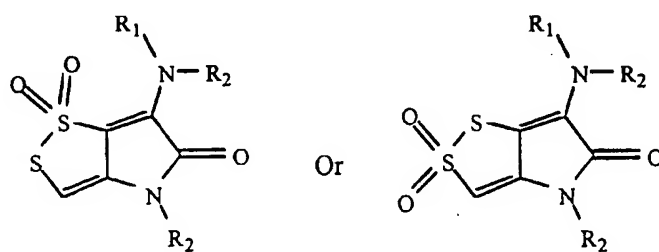


Figure 3

INTERNATIONAL SEARCH REPORT

Intern. Application No

PCT/CA 98/00841

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/40 C07D495/04 //C12P17/18, C07D495/04, (C07D495/04,
339:00, 209:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K C07D C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SHARMA S ET AL: "SCREENING OF POTENTIAL CHEMOPREVENTIVE AGENTS USING BIOCHEMICAL MARKERS OF CARCINOGENESIS" CANCER RESEARCH, vol. 54, no. 22, 15 November 1994, pages 5848-5855, XP000575882 see abstract see entry "Thiolutin" in Table 1 on page 5852 see page 5852; table 1 see page 5849, column 1, line 5, paragraph 1	1-18
A	WO 84 01775 A (BIOTECH AUSTRALIA PTY LTD ;COMMW SCIENT IND RES ORG (AU)) 10 May 1984 see page 2 - page 5; claims	1-18

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

5 January 1999

Date of mailing of the international search report

15/01/1999

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

Paisdor, B

INTERNATIONAL SEARCH REPORT

Intern: al Application No

PCT/CA 98/00841

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WO 96 32396 A (WEBSTER JOHN MALCOLM ;LI JIANXIONG (CA); CHEN GENHUI (CA)) 17 October 1996 cited in the application see abstract; claims -----</p>	1-18

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA 98/00841

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 10-18
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 10-18
are directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 98/00841

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 8401775 A	10-05-1984	AU 558287 B CA 1214130 A EP 0126092 A US 4672130 A ZA 8307974 A	22-01-1987 18-11-1986 28-11-1984 09-06-1987 26-09-1984
WO 9632396 A	17-10-1996	AU 5264796 A CA 2217957 A	30-10-1996 17-10-1996